REMARKS

I. INTRODUCTION

Claims 31 and 39 have been amended. Claims 1-30 and 34-35 were previously cancelled. Claims 41-67 were withdrawn. Thus, claims 31-33 and 36-67 are pending in the present application. No new matter has been added. In view of the above amendments and the following remarks, it is respectfully submitted that claims 31-33 and 36-40 are in condition for allowance.

II. THE RESTRICTION REQUIREMENT SHOULD BE WITHDRAWN

The Examiner responds to the arguments presented relating to the Restriction Requirement. (See 3/19/09 Office Action, p. 14). Although the Examiner again does not explicitly re-present the restriction requirement, Applicants maintain the position that the claims are of a single general inventive concept under PCT Rule 13.1. Applicants again respectfully maintain all of the previously presented arguments regarding withdrawal of the restriction requirement.

The Examiner asserts that the common technical feature among the different purported groups is the culture media. The Examiner further states that the intended use of the composition for the expansion of stem cells is not a feature of all the groups. Regarding the intended use, it is respectfully submitted that the intended use is not necessarily the common technical feature among the groups. In fact, the Examiner seems to agree that the culture media is the common feature. The culture media itself is what provides the improved results of using the culture media of the present invention for expanding autologous human stem cells to be realized. The Examiner further stated that in view of Xia, the claimed composition of the present invention is merely an obvious variant and differs only in the concentration of the heparin component. As discussed with regard to the further rejections presented by the Examiner, heparin may be a result effective variable. However, those skilled in the art will understand that this only holds true when concerning the intended use of the media as taught in Xia. A basic difference between the media of Xia and the present invention is in the intended use of the culture media. Depending on the use of the media, a variety of different components may be a result effective variable.

In response to the above argument, the Examiner states that "the intended use of the claimed composition only requires that the composition be in a form suitable for this intended use." (See 8/18/09 Office Action, p. 14). As stated above, it is again maintained that the culture media is what provides the improved results for expanding autologous human stem cells. That is, as discussed above, the intended use is not necessarily the common technical feature among the groups. In addition, it is respectfully submitted that in the field of art in which the present invention belongs, those skilled in the art will understand the importance of concentration levels of the different components of the culture media. That is, despite one culture media having common components as another culture media, it is wholly possible that the concentrations may result in opposing results (*e.g.*, promoting expansion versus preventing expansion). Thus, merely being a form "suitable" may not be enough to realize a different intended use.

The Examiner further states that "the concentration of all components in any culture media composition, regardless of the intended use, are result effective variables since they affect the visible result of cell viability and growth." (See Id.). It appears that the Examiner agrees with the above argument that concentration levels are of utter importance, in particular with culture media in the present field of art. Therefore, it is unclear why the Examiner appears to state that a composition merely having a form that is suitable is enough to meet a limitation when the Examiner also appears to understand that all components of any culture media are "result effective variables." That is, the components of a culture media are dependent upon both type *and* concentration to realize an intended use.

Previously, it was argued that the Examiner stated that the method steps for using a claimed composition is irrelevant unless they require a structural form or feature to be present or absent in the claimed composition. Again, the Examiner resorts to the basis that the media composition of Xia and the present invention are used for the generic autologous human progenitor cells. It is respectfully maintained that, in view of the amendments submitted herewith and the explanations below, the structural form is a feature and thus, present in the claimed composition.

In response to this argument, the Examiner states that "the Xia reference is silent with regard to the manner in which the serum component was collected in the reference method and any suggestions that other methods of collection of the serum were more likely are irrelevant given that the Duggins reference provides evidence that plasmapheresis is commonly used to obtain serum proteins and to produce cell culture media." (See 8/18/09 Office Action, p. 5). The Examiner previously states that a type of cell to which a media is used is irrelevant to the examination of the composition *unless* they require a structural form or feature to be present or absent. (See 3/19/09 Office Action, p. 4). It was previously argued that the structure of the serum in Xia is indeed different from the structure of the autologous serum of the present invention. The previous argument arose from the Examiner's statement that a type of cell to which a composition is to be used is reliant on a structural form or feature. The structure of the autologous serum is again being stated to be of importance and different than the serum of Xia. It is unclear why the Examiner states in a first instance a case where the structural form is important for distinguishing to which a reply was provided. Yet, in the present Office Action, the Examiner appears to state that compositions do not have to be structurally identical and may include further components. However, those skilled in the art will understand that additional components or fewer components may drastically alter a structure of the serum to a point where the composition is incapable to be used with the intended type of cell. Consequently, only a common structure would have a "sufficient motivation and a reasonable expectation of success" to those skilled in the art. That is, a change in structure would result in one skilled in the art to conclude no reasonable expectation of success.

III. THE OBJECTION TO THE CLAIM SHOULD BE WITHDRAWN

The Examiner has objected to claim 31 for an informality. (See 8/18/09 Office Action, p. 2). Specifically, claim 31 includes a typographical error. The incorrectly spelled word has been amended according to the Examiner's suggestion.

IV. THE 35 U.S.C. § 112 REJECTION SHOULD BE WITHDRAWN

The Examiner has rejected claims 31-33 and 36-40 under 35 U.S.C. § 112, first paragraph, for failing to comply with the written description requirement. (See 8/18/09 Office Action, p. 3).

The Examiner rejects claims 31 and 39 for having a limitation of "0.001 and 100 mg/ml" of protamine which purportedly does not have sufficient support in the disclosure as originally filed. Specifically, the Examiner states that the disclosure only recites 0.1 and 10,000 UI/ml on p. 3 of the Specification. In response to the 3/19/09 Office Action and in an attempt to expedite processing of the present application by using units that would coincide with a prior art reference, claim 31 was amended to use concentration units that the prior art also uses as a measure of protamine. The conversion was based upon a coefficient that would properly amend the UI/ml of protamine to the mg/ml equivalent without exceeding said amount. However, in view of the Examiner now rejecting claims 31-33 and 36-40 under 35 U.S.C. § 112, first paragraph, claims 31 and 39 have been amended to recite the originally listed values using the UI/ml units.

The Examiner previously stated that since "the prior art measures protamine in concentration units of mg/ml which is contrary to Applicant's disclosure of UI/ml the metes and bounds of the claim are unclear." (See 3/19/09 Office Action, p. 12). So that the Examiner is not forced to repeat the prior rejection on similar grounds, this amendment will be addressed herein. It is respectfully submitted that the concentration units disclosed in the prior art is not the only standard in which to provide concentration units. The unit UI represents "International Unit" which those skilled in any scientific art would understand. Furthermore, anyone skilled in the art would understand that concentration units, or any similar units, may be converted into other forms of units. As an example, if a prior art reference discloses a weight in "pounds" while a claim discloses a mass in "grams," those of skill in the art would not be unclear as to the relationship between these two units. That is, "grams" would not have metes and bounds that are unclear. In fact, the above example relates to a weight and a mass which technically are not even a common type of unit. Yet, those skilled in the art would not be confused. It is also unclear why the Examiner would state that a concentration unit of mg/ml is *contrary* to a concentration unit of UI/ml. The term concentration itself merely refers to an amount per unit volume which both units indicate. Thus, claims 31 and 39 have been amended to revert back to the UI/ml standard as those skilled in the art would fully understand and be clear as to the relationship between UI and mg relating to protamine.

Accordingly, it is respectfully submitted that the range of "0.1 and 10,000 UI/ml protamine" is sufficiently described in the Specification at the time the application was filed as well as being sufficiently clear to one skilled in the art without providing a full discourse on the proper conversion to a different standard used in another disclosure. Therefore, it is respectfully submitted that the Examiner should withdraw the 35 U.S.C. § 112 rejection for claims 31 and all dependent claims 32-33, 36-40.

V. THE 35 U.S.C. §§ 102(b), 103(a) REJECTIONS SHOULD BE WITHDRAWN

The Examiner rejects claims 31-33 and 36-37 under 35 U.S.C. §§ 102(b), 103(a) as unpatentable over The Journal of Immunology, Xia et al., 2002 (hereinafter "Xia") in view of U.S. Pat. No. 4,735,726 to Duggins. (See 3/19/09 Office Action, p. 14).

Claim 31 has been amended to recite an autologous expansion culture medium of autologous human progenitor stem cells comprising "between 0.1% and 90% weight of autologous human serum supplemented with between 0.1 and 10,000 UI/ml heparin and between 0.1 and 10,000 UI/ml protamine," and "a base culture medium including basic nutrients wherein the autologous human serum is obtained by plasmaphersis with heparin and protamine."

The Examiner states that the autologous serum is deemed to be met by Xia. (See 8/18/09 Office Action, p. 6). The Examiner also states, even if this were not the case, obtaining autologous human serum from the blood of a patient by means of plasmapheresis is a well established and conventional procedure and therefore obvious as purportedly evidenced by Duggins. (See Id.). Initially, it appears that the use of Duggins is to supplement or provide inherency that plasmapheresis is a commonly used process to obtain serum proteins and to produce cell culture media. It also appears that the Examiner assumes that the structure of the serum in Xia and the autologous serum of claim 31 would be structurally the same simply from the concentrations of the autologous serum, heparin, and the protamine. However, it is respectfully submitted that the structure of the autologou serum of present invention involves more than just the components thereof. Thus, the previously submitted arguments relating to the structure of the autologous serum is maintained.

In particular, as was previously argued, it is respectfully submitted that the structure of the autologous serum of the present invention is different from that of Xia. The structure (chemical composition) of the autologous serum obtained by plasmapheresis with heparin/protamine is unlike any obtained by other procedures, including plasmapheresis with Anticoagulant Citrate Dextrose Solution (ACD). One skilled in the art may obtain autologous serum manually, for example, by drawing blood from the patient with a syringe or mechanically by plasmapheresis. The most common way of obtaining autologous serum is the manual drawing of blood from the patient. While the serum isolating procedure is not specified in Xia, those skilled in the art may assume that that the serum was obtained in this manner. Among the instructions for manually drawing blood from the patient and isolating the serum in the drawn blood is allowing the blood to coagulate followed by centrifugation and microfiltration. The coagulation of blood requires the release of coagulation factors from the patelets. These factors remain soluble in the filtered serum and are inevitably present in the culture media used in laboratory research, such as that of Xia. Those skilled in the art will understand that serum allowed to clot naturally stimulates cell proliferation more than serum from which cells have been removed physically, to which patelet released factors account for this effect. Accordingly, the structural difference between the serum used in Xia and the serum of the present invention is a technical characteristic of the serum used to complement the media that affects cell physiology and accounts for a difference in the outcome of the cell culture exposed to the serum.

Also as was previously argued, in an alternate embodiment, manually drawn blood may be treated with an anticoagulant. Xia explains that in order to isolate monocytes from healthy donors, venous blood is drawn and anticoagulated with a 3.8% sodium citrate solution. Thus, in the event that any assumption regarding the use of anticoagulants for obtaining autologous serum were to be made, it should be that sodium *citrate* is used as the anticoagulant. Autologous serum obtained by drawing blood manually with the use of sodium citrate as the anticoagulant is also structurally different from the serum used in the expansion media of the present invention, as will be explained in further detail below.

In the plasmapheresis procedure, whole blood is continuously removed from the patient through a central venous catheter and enters the pheresis machine through an extracorporeal

circuit. Within the machine, cells are immediately separated from plasma by filtration, suspended in replacement fluid and promptly returned to the subject's body, while plasma is put to its desired use. Despite no time being allowed for clotting, using an anticoagulant is highly recommended in plasmapheresis to allow the flow of blood from the patient's body to the pheresis machine, where commonly used anticoagulants include heparin and ACD. Once the plasmapheresis procedure is finished, protamine is given to the patient in order to neutralize heparin and allow for the normal physiology of reconstructed blood. A neutralizing dose of protamine will be added to the acellular filtered plasma. Then, plasma proteins will be allowed to clot, due to the presence of calcium, and cleared plasma retrieved by centrifugation. It is noted that acellular plasma protein coagulation does not involve the releasing of coagulating factors. This structural difference allows an optimal physiological environment for the cells being expanded for autologous drafting purposes.

Those skilled in the art will understand the biochemical basis for the anticoagulating action of ACD being the chelating of calcium by citrate. Acellular clotting of proteins will not occur in the absence of calcium, therefore the plasma obtained by plasmaphareris with ACD is structurally different from the plasma obtained by plasmapheresis with heparin/protamine because it contains additional proteins or increased concentration of proteins that may affect the fate of cells being expanded for autologous drafting purposes. For example, such proteins may act as adyuvants in the immune response to transplant rejection. Moreover, calcium is a signaling molecule for cell attachment, cell growth, and differentiation. The centrally positioned signaling molecule Ras is very sensitive to calcium levels. This small GTPase operates as a binary molecular switch and regulates cell proliferation and differentiation. A cell in culture decodes a variety of $InsP_3$-dependent Ca^{2+} signals in time, amplitude, and space during the process of cardiac cell differentiation and heart development. Studies performed in embryonic stem cell differentiating in cardiomyocytes have uncovered that Ca^{2+} regulates multiple steps of cell differentiation. These include secretion of cardiogenic factors, cardiac transcriptional cascades and in turn gene expression, myofibrillogenesis, and initiation of embryonic pacemaker activity. Thus, Ca^{2+} is a major second messenger directing the fate of stem cells. Its absence, due to the presence of chelating factors like citrate, undoubtedly affects the differentiation of

progenitor stem cells and their gene expression which may lead to immune rejection upon transplantation.

Thus, it is respectfully submitted that the structure of the autologous serum specified in the expansion media as recited in claim 31 confers a special technical feature to the expansion media such that it results optimally for expanding progenitor-stem cells for autologous drafting purposes, in particular with regards to the structure of the autologous serum. Accordingly, at the time the present application was filed, the media of the present application constitutes an alternative media suitable for expanding progenitor stem cells, in particular muscle progenitor stem cells.

Regarding the Examiner's assertion that even if the concentrations were not the same, "one of ordinary skill in the art would have been motivated to modify the concentration of the heparin either up to enhance the results of the heparin on the cells or down in order to save money and conserve resources." (See 8/18/09 Office Action, p. 7). Regarding the concentrations, please note the above arguments related to the Restriction Requirement. Furthermore, please note the amended argument relating to saving money and/or conserving resources.

Due to the translation error that was corrected in the Amendment for the 3/19/09 Office Action, it is respectfully submitted that the motivation indicated by the Examiner is in fact opposite to what the present invention would reasonably provide. As was amended, the heparin concentration may reach up to 10,000 UI/ml. In contrast, Xia includes data in which the heparin concentration never deviates from 25 UI/ml. That is, the concentration of heparin may potentially be 400 times the amount as disclosed in Xia. Thus, this would not lead one skilled in the art to reasonably conclude that resources are conserved nor are costs lowered.

For at least the above described reasons, it is respectfully submitted that neither Xia nor Duggins, either alone or in combination, does not disclose or suggest the recitation of claim 31. Accordingly, it is respectfully submitted that claim 31 is allowable and the Examiner should withdraw the 35 U.S.C. §§ 102(b), 103(a) rejection for this claim. Because claims 32, 33, 36,

and 37 depend from and, therefore, include the limitations of claim 31, it is respectfully submitted that these claims are also allowable.

VI. THE 35 U.S.C. § 103(a) REJECTIONS SHOULD BE WITHDRAWN

The Examiner rejects claims 31-33 and 36-40 under 35 U.S.C. § 103(a) as unpatentable over U.S. Pat. Pub. No. 2002/0124855 to Chachques in view of U.S. Pat. No. 7,015,037 to Furcht et al. (hereinafter "Furcht") in further view of U.S. Pat. No. 4,735,726 to Duggins in further view of U.S. Pat. No. 6,624,141 to Yang et al. (hereinafter "Yang"). (See 8/18/09 Office Action, p. 8).

The Examiner maintains the previous rejection in view of the above references. (See 8/18/09 Office Action, p. 8). Specifically, the Examiner states that the culture medium of Chachques emphasizes the importance of avoiding an immune response by using autologous cells and Furcht teaches that cardiac cells can be cultured with autologous serum. (See 8/18/09 Office Action, p. 16). The previously presented arguments are again being maintained. Furcht explicitly addresses the solutions for preventing immune rejection by disclosing specific approaches for transplantation to prevent immune rejection. (See Furcht, col. 28, l. 61 – col. 29, l. 25). In this discussion, Furcht *does not include* the use of autologous serum. Due to the detail involved in the description of this section, one skilled in the art would reasonably assume that autologous serum would have no effect on the outcome of cells expanding for drafting purposes in view of the teachings of Furcht. Thus, the likelihood of adding autologous serum to the media is not a reasonable combination between Chacques and Furcht. Furthermore, despite using plasmapheresis with heparin/protamine, the medium of the present application is not necessarily obvious. Despite one in the art potentially reaching the solution in order to provide for an alternative culture media that diminishes rejection upon transplantation (which is not conceded), the skilled person in the art would not have decided to combine the teachings in the cited documents as a solution to the problem of avoiding rejection.

Thus, for the reasons discussed above, it is respectfully submitted that the cited references do not obviate the recitation of claim 31. Accordingly, it is respectfully submitted that claim 31 is allowable and the Examiner should withdraw the 35 U.S.C. § 103(a) rejection for

this claim. Because claims 32, 33, and 36-40 depend from and, therefore, include the limitations of claim 31, it is respectfully submitted that these claims are also allowable.

The Examiner rejects claims 31-33 and 36-40 under 35 U.S.C. § 103(a) as unpatentable over U.S. Pat. No. 6,472,212 to Valerio et al. (hereinafter "Valerio") in view of U.S. Pat. No. 5,817,773 to Wilson et al. (hereinafter "Wilson") in further view of Duggins. (See 3/19/09 Office Action, p. 11). Initially, it is noted that the change to this rejection is based on Duggins purportedly supplementing that plasmapheresis is a commonly used process to obtain serum proteins and to produce cell culture media. The Examiner appears to maintain the previous rejection for several reasons. (See 8/18/09 Office ction, pp. 17-18).

It was previously argued that those skilled in the art will understand that in the field that bone marrow cells and, in general, cells of the blood lineage, do not need attachment to substrates in order to be expanded. That is, these cells grow in *suspension*. This is an important property or technical character that is taken into account when designing media composition because culture media designed for suspension-growing cells may not support attachment dependent growing cells. The Examiner responds by stating that modifying a timing of the addition of supplements is a matter of routine optimization and experimentation, thereby rendering a timing issue obvious. However, as discussed above, the issue of intended use is revisited. Because the field of bone marrow cells or cells of the blood lineage do *not* need attachment to substrates in order to be expanded, one skilled in the art would *not* reasonably be led to the purportedly obvious conclusion that the Examiner states otherwise. Those skilled in the art will understand that progenitor stem cells are attachment-dependent. This limitation is a technical characteristic of the present invention. Culture media that does not allow attachment of cells or permits only poor attachment of cultured cells to the plate surface will result in a high rate of detachment and death of expanding progenitor stem cells, thereby precluding expansion to cell counts suitable for transplantation. The claimed media composition presents high autologous calcium content which is critical for cell attachment. The presence of autologous calcium in the media is a consequence of the novel combination of media components as that recited in claim 31. Specifically, as described above, the autologous serum chosen by its

isolation method (*i.e.*, plasmapheresis with heparin/protamine) results in high levels of autologous calcium in the final media composition.

It was previously noted that it appears that the Examiner is constructing the claimed media by adding unconnected references solely on the basis that they contain the element that is suitable to obtain the claimed media regardless of the functionality that component may provide the respective reference. Thus, it was respectfully submitted that the functionality of the component is a critical factor that is part of the culture media and that the Examiner's attempt at randomly selecting references to obviate claim 31 is impermissible hindsight. The Examiner responds to this by stating that "so long as [the reconstruction] takes into account only knowledge which was within the level of ordinary skill at the time the claimed invention was made, and does not include knowledge gleaned only form the applicant's disclosure, such a reconstruction is proper." (See 8/18/09 Office Action, p. 17, citing *In re McLaughlin*, 443 F.2d 1392, 170 USPQ 209 (CCPA 1971)). However, in light of the above arguments, in particular those concerning the fields of endeavor of the prior art references and the concentration dependency of the autologous serums, it is respectfully maintained that the Examiner is constructing the claimed media through impermissible hindsight.

The Examiner also states that the argument regarding the order in which the components of the media being critical is merely argument of counsel and unsupported. (See 8/18/09 Office Action, p. 18). This argument is respectfully being maintained and is not merely argument of counsel as it is based on objective evidence. As discussed previously, it is not reasonable to add the histamine to the protamine-containing medium of Valerio because the medium was designed to have a particular physiological effect on the growing cells. This physiological effect is a technical characteristic of the media and it is provided by its protamine content. Those skilled in the art would understand that a selection of a starting media with technical characteristics that are suitable for solving the problem is critical in attempting to find a final media. To subsequently neutralize the technical characteristic that was specifically selected for the trials would run contrary to basic experimentation principles. In the interest of objectivity (thus the present Specification is not noted), these principles are founded at least in the prior art references listed

in ¶ [0006] as well as in Xia which discusses lengths of time in which components are added and further components being added thereafter (See Xia, p. 1132).

Thus, for at least the above reasons, it is respectfully submitted that claim 31 is allowable and the Examiner should withdraw the 35 U.S.C. § 103(a) rejection for this claim. Because claims 32, 33, and 36-40 depend from and, therefore, include the limitations of claim 31, it is respectfully submitted that these claims are also allowable.

CONCLUSION

In light of the foregoing, Applicants respectfully submit that all of the now pending claims are in condition for allowance. All issues raised by the Examiner having been addressed, and an early and favorable action on the merits is earnestly solicited.

Respectfully submitted,

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